Supporting information

Revisiting Monosaccharide Analysis – Quantitation of a Comprehensive Set of Monosaccharides using Dynamic Multiple Reaction Monitoring

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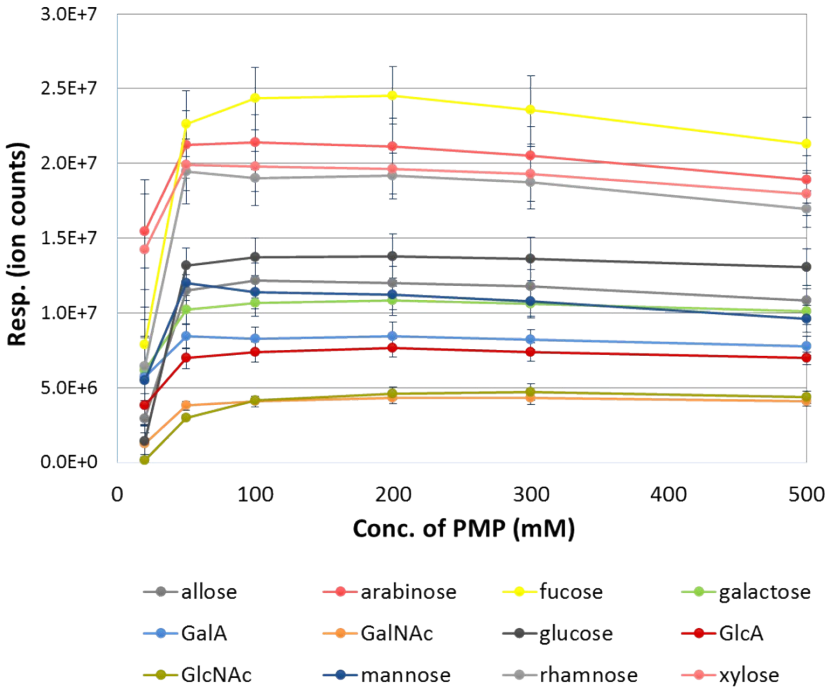

Figure S1. Optimization of PMP concentration

Figure S2

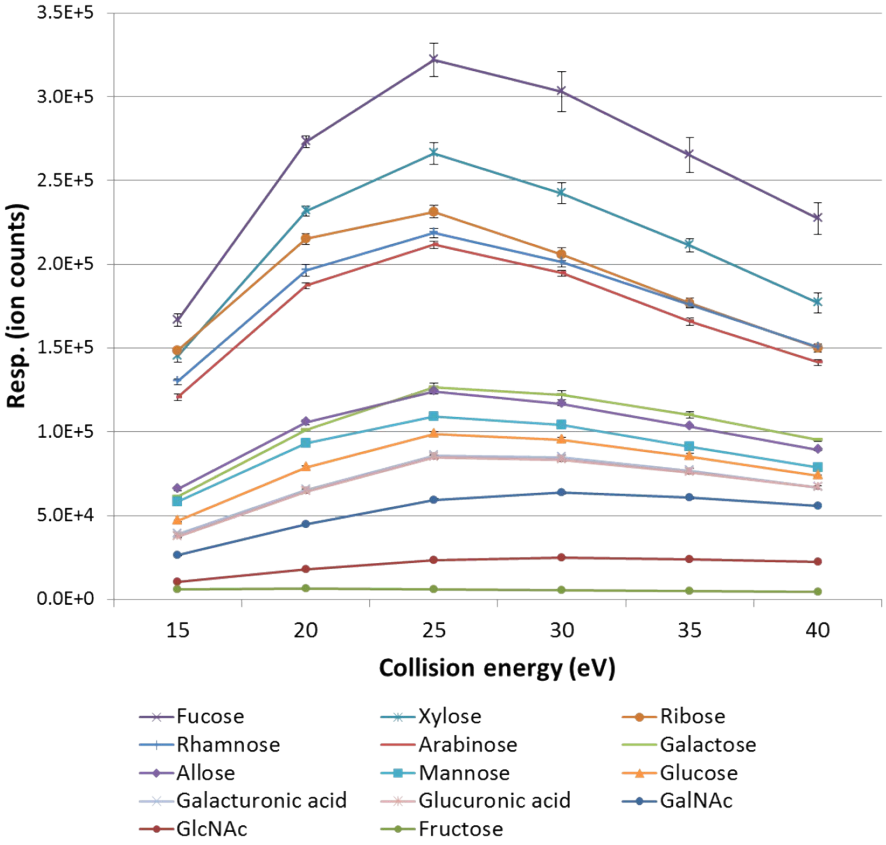

Figure S2. Optimization of collision energy

Figure S3

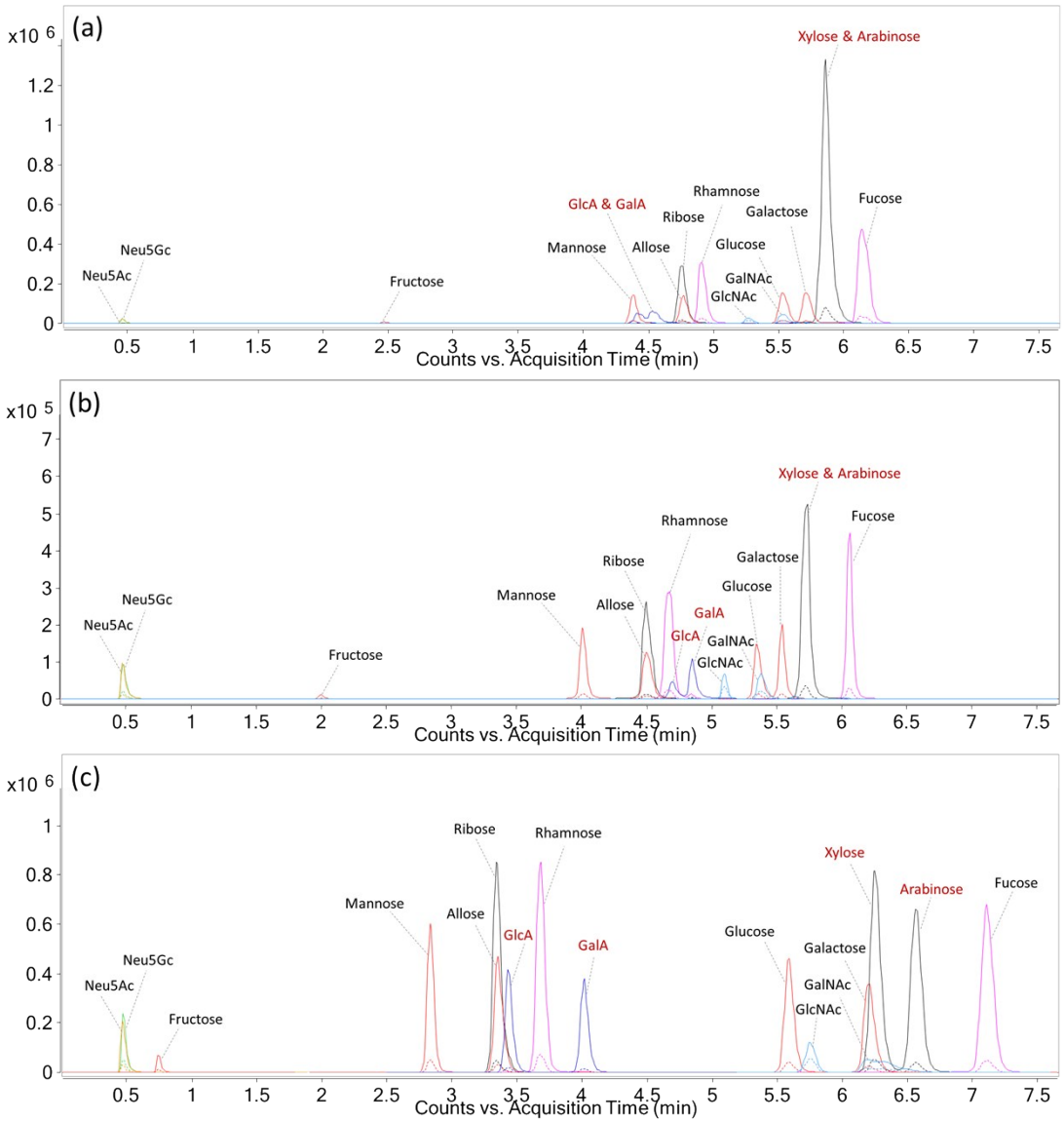

Figure S3. Optimization of mobile phase A and UHPLC gradient for isomer separation. (a) No buffer, with gradient of 0.0-8.0 mins, 5-30% B; 8.1-9.0 mins, 99% B; 9.1-10.0 mins, 5% B; (b) 25 mM ammonium acetate at pH 7, with gradient of 0.0-8.0 mins, 5-25% B; 8.1-9.0 mins, 99% B; 9.1-10.0 mins, 5% B; (c) 25 mM ammonium acetate at pH 8.2, with gradient of 0.0-7.0 mins, 12-15% B; 7.1-8.5 mins, 99% B; 8.6-10.0 mins, 12% B.

Table S1. Comparison of different methods for monosaccharide quantitation.[1-4]

Method	GC-FID	CE-UV	HPAEC-PAD	LC-MS (ion trap)	UPLC-QqQ
Reference	Wang et al., 2017	Hu et al., 2014	Zhang et al., 2012	Rühmann et al., 2014	This study
Number of monosaccharides analyzed	9	10	16	15	16-25
Derivatization (hours)	> 8.5	0.5	0	1	0.5
Need complete baseline separation	Yes	Yes	Yes	No	No
Analysis time (min/run)	60 min	≥ 20 min	> 40 min	12 min*	10 min
Structural information	No	No	No	Yes	Yes
Interference	Little	UV-absorbing compounds	amino acids and peptides	Little	Little
Limit of detection (pmol)	≥ 1.5	≥ 9	~12	≥ 0.7	0.000056-0.016
Linear range (orders of magnitude)	two to three	one to two	two	two to three	four to six
Precision (CV%)	0.5-4.3	1.1-2.7	N/A	1.2-3.8	1.0-7.2

*Compounds were separated into two standard solutions for method development and validation. Xylose and arabinose were not resolved.

Table S2. Complete list of monosaccharide MRM transitions

Compound	Mass	MRM transitions		Collision energy (eV)
		Precursor ion	Product ions	
D-Ribose	150.1	481.2	**175.0**, 217.1	25
D-Xylose	150.1	481.2	**175.0**, 217.1	25
D-Arabinose	150.1	481.2	**175.0**, 217.1	25
D-Apiose	150.1	481.2	**175.0**, 217.1	25
D-Lyxose	150.1	481.2	**175.0**, 217.1	25
L-Rhamnose	164.2	495.2	**175.0**, 217.1	25
L-Fucose	164.2	495.2	**175.0**, 217.1	25
D-Fructose	180.2	511.2	**175.0**, 217.1	20
D-Mannose	180.2	511.2	**175.0**, 217.1	25
D-Allose	180.2	511.2	**175.0**, 217.1	25
D-Glucose	180.2	511.2	**175.0**, 217.1	25
D-Galactose	180.2	511.2	**175.0**, 217.1	25
D-Altrose	180.2	511.2	**175.0**, 217.1	25
D-Talose	180.2	511.2	**175.0**, 217.1	25
L-Gulose	180.2	511.2	**175.0**, 217.1	25
D-Glucuronic acid	194.2	525.2	**175.0**, 217.1	25
D-Galacturonic acid	194.2	525.2	**175.0**, 217.1	25
D-Glucosamine	179.2	510.2	**175.0**, 216.1	25
D-Galactosamine	179.2	510.2	**175.0**, 216.1	25
D-Mannosamine	179.2	510.2	**175.0**, 216.1	25
GlcNAc	221.2	552.2	**175.0**, 216.1	30
GalNAc	221.2	552.2	**175.0**, 216.1	30
ManNAc	221.2	552.2	**175.0**, 216.1	30
Neu5Ac	309.3	310.3	**274.2**, 167.1	5
Neu5Gc	325.3	326.3	**290.2**, 167.1	5

Figure S4

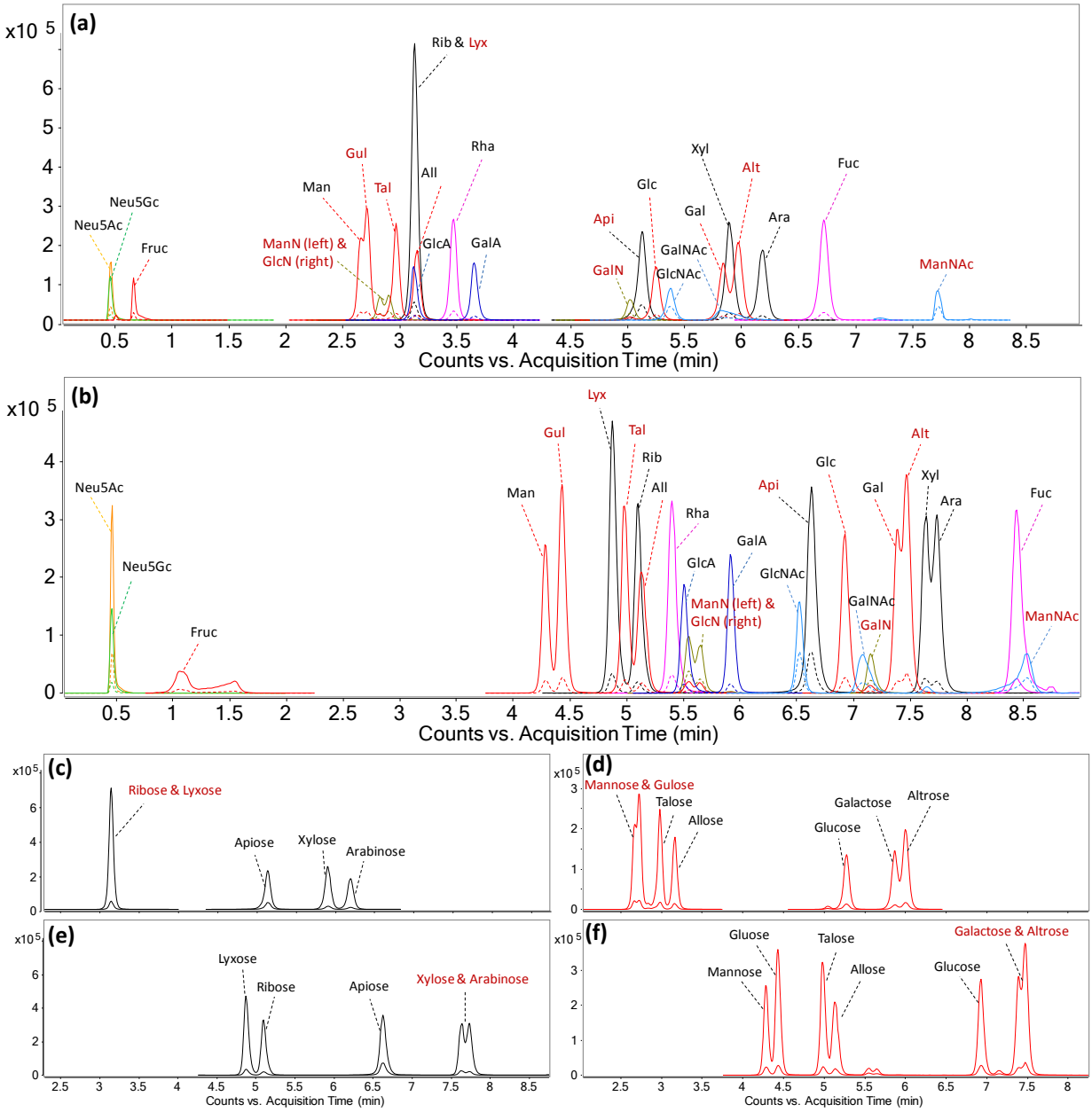

Figure S4. Separation of more isomers under different UHPLC conditions using 25 mM ammonium acetate buffered solvent A. (a) Optimized gradient at pH 8.2; (b) Optimized gradient at pH 7.0; (c) Separation of pentose isomers at pH 8.2; (d) Separation of hexose isomers at pH 8.2; (e) Separation of pentose isomers at pH 8.2; (f) Separation of hexose isomers at pH 8.2.

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